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EXPRESS MAIL LABEL NO. EL828
DATE OF DEPOSIT:

JC17 Rec'd PCT/PTO 29

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUM
5585-59367

U S APPLICATION NO (If known, see
09/8695

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/00003	6 January 2000	6 January 1999

TITLE OF INVENTION
WOUND HEALING AND OROFACIAL CLEFTING

APPLICANT(S) FOR DO/EO/US
Alexander Fred Markham and David Bonthron

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. § 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau
   c ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))
   a ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C § 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 and the Recordal fee of $40.00 is included.
13. ☒ A **FIRST** preliminary amendment.
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:
    ☐ Written Opinion.
    ☐ Preliminary Examination Report.
    ☐ International Search Report.
    ☐ Copies of References Cited.

24197

JC18 Rec'd PCT/PTO 29 JUN 2001

EXPRESS MAIL LABEL NO. EL828141

DATE OF DEPOSIT: June 29,

U.S. APPLICATION NO (If known, see 37 C.F.R. § 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/869564	PCT/GB00/00003	5585-59367

17. ☒ The following fees are submitted:

	CALCULATIONS (PTO USE ONLY)
BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):	
Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**	
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO **$710.00**	
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$	860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	44 - 20 =	24	x $18.00	$	432.00
Independent Claims	12 - 3 =	9	x $80.00	$	720.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	270.00
TOTAL OF ABOVE CALCULATIONS =				$	2,282.00
☒ Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.				$	1,141.00
SUBTOTAL =				$	1,141.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	1,141.00
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). **$40.00** per property. +				$	
TOTAL FEES ENCLOSED =				$	1,141.00
				REFUND →	$
				CHARGE →	$

a. ☒ A check in the amount of $ 1141.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No. 02-4550. A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received.

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KLARQUIST SPARKMAN CAMPBELL
LEIGH & WHINSTON, LLP
One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, OR 97204-2988

William D. Noonan

SIGNATURE

William D. Noonan, M.D.
NAME

30,878
REGISTRATION NUMBER

cc: Docketing

09/869564

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PRIORITY Rec'd 19 OCT 2001

In re Application of: Markham et al.

Art Unit:

Application No. 09/869,564

Filed: June 29, 2001

For: WOUND HEALING AND OROFACIAL CLEFTING

Examiner: not yet assigned

Date: October 1, 2001

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on October 1, 2001 as First Class Mail in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

William D Noonan

Attorney for Applicant

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SECOND PRELIMINARY AMENDMENT

Please amend the Sequence Listing as follows:

Please replace pages 1-22 of the sequence listing submitted on August 1, 2001, with the sequence listing submitted herein, pages 1-25.

Please amend the claims as follows:

1. (Amended) An isolated nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridises to a nucleic acid sequence shown in SEQ ID NO: 1 under high stringency conditions.

2. (Amended) The isolated nucleic acid molecule of Claim 1 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

3. (Amended) The isolated nucleic acid molecule of claim 1 or claim 2 wherein the nucleic acid molecule is from a mammal.

4. (Amended) The isolated nucleic acid molecule of Claim 3 wherein the mammal is a human.

5. (Amended) A method for diagnosing orofacial clefting in a patient, comprising detecting expression of a nucleic acid sequence shown in SEQ ID NO:1, or fragments or variants of the nucleic acid sequence shown in SEQ ID NO:1, in selected target tissues(s).

6. (Amended) A method for diagnosing orofacial clefting in patients suffering from, or suspected to be suffering from orofacial clefting, comprising detecting a mutation in a nucleic acid sequence shown in SEQ ID NO:1, or fragments or variants of the nucleic acid sequence shown in SEQ ID NO:1.

7. (Amended) A polypeptide or a protein comprising an epitope for an antibody, or a protein modified by one or more amino acid modifications and comprising an epitope, or a modified or unmodified fragment comprising an epitope for a tissue repair protein encoded by a nucleic acid sequence having at least 75% identity to a nucleic acid sequence shown in SEQ ID NO:39.

8. (Twice Amended) A delivery vehicle comprising the nucleic acid molecule of Claim 1 and/or the polypeptide of Claim 7, which is in the form of a suspension.

9. (Amended) The delivery vehicle of Claim 8, wherein the delivery vehicle is adapted to deliver the nucleic acid molecule or polypeptide to a selected tissue.

10. (Amended) An antibody raised against the polypeptide of Claim 7.

11. (Amended) The antibody of Claim 10 wherein the antibody is a monoclonal antibody.

12. (Amended) A method for diagnosing orofacial clefting, comprising detecting expression of a protein recognized by the antibody of claim 10.

13. (Amended) A method for detecting the antibody of claim 10 in a sample, comprising:

labeling a ligand comprising a protein or protein fragment of SEQ ID NO:2 present in the sample;

contacting the sample with the antibody of claim 10, wherein the antibody is immobilised which results in binding of the immobilised antibody to the labelled ligand; and

detecting the labelled ligand bound to the immobilised antibody in the sample.

14. (Twice Amended) A method for the treatment of orofacial clefting, comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule of Claim 1 and/or polypeptide of Claim 7.

15. (Twice Amended) A method for treating wounds and/or promoting tissue repair, comprising administering to a patient suffering from a wound and/or tissue damage the nucleic acid molecule of Claim 1 and/or the polypeptide of Claim 7.

16. (Twice Amended) A method of treating wounds and/or promoting tissue repair, comprising administering to a patient suffering from a wound and/or tissue damage a composition comprising the delivery vehicle of Claim 8.

17. (Twice Amended) A pharmaceutical composition comprising the nucleic acid of Claim 1 and/or the protein of Claim 7.

18. (Twice Amended) A method for treating orofacial clefting and/or wound healing and/or tissue repair comprising administering to a patient the nucleic acid of Claim 1 and/or the protein of Claim 7.

19. (Amended) An isolated nucleic acid molecule encoding a tissue repair protein comprising a nucleotide sequence which hybridises to a nucleic acid sequence shown in SEQ ID NO:3 under high stringency conditions.

20. (Amended) The isolated nucleic acid of Claim 19 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

21. (Twice Amended) The isolated nucleic acid of Claim 19 wherein the nucleic acid molecule is from a mouse.

22. (Amended) The polypeptide or a protein of claim 7, wherein the nucleic acid sequence comprising at least 75% identity to the nucleic acid sequence shown in SEQ ID NO:39 comprises a nucleic acid sequence shown in SEQ ID NO:3, or a fragment thereof.

23. (Twice Amended) A delivery vehicle comprising the isolated nucleic acid molecule of Claim 20 and/or the polypeptide of Claim 22.

24. (Amended) An antibody raised against the polypeptide of Claim 22.

25. (Amended) The antibody of Claim 24 wherein the antibody is a monoclonal antibody.

26. (Twice Amended) A method for diagnosing or detecting orofacial clefting comprising detecting expression of a protein recognized by the antibody of claim 24.

27. (Amended) A method of producing a transgenic mammal comprising disrupting a gene, or the effective part of the gene, wherein the gene encodes at least one tissue repair protein, and a resulting phenotype is a cleft palate in the transgenic mammal.

28. (Amended) The method of Claim 27 wherein the transgenic mammal is a rodent.

29. (Twice Amended) The method of Claim 28 wherein the rodent is a mouse.

30. (Twice Amended) The method of Claim 27 wherein the gene encoding the tissue repair protein is a nucleic acid molecule comprising a nucleotide sequence which hybridizes to a nucleic acid sequence shown in SEQ ID NO: 3 under high stringency conditions.

31. (Amended) The method of Claim 27 wherein the transgenic mammal is a human.

32. (Twice Amended) The method of Claim 27 wherein the gene encoding the tissue repair protein is a nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridizes to a nucleic acid sequence shown in SEQ ID NO: 1 under high stringency conditions.

33. (Amended) A reporter gene construct based on a promoter region of a gene, or an effective part thereof encoded by SEQ ID NO:1, or a fragment or variant thereof.

34. (Amended) A method for detection/screening a pharmaceutical and/or other compound, comprising:

contacting the pharmaceutical and/or other compound with a reporter gene construct of claim 33; and

determining a transcriptional response of the reporter gene to the pharmaceutical or other compound wherein the transcriptional response is an indicator of a potential teratogenic effect of the pharmaceutical or other compound.

35. (Reiterated) A cloned nucleic acid molecule encoding a tissue repair protein contained in a Yeast Artificial Chromosome species designated as AB 1380 YAC-CP 1 and deposited with NCIMB Limited of Aberdeen, Scotland (UK) under accession number NCIMB 41005.

36. (Amended) An isolated nucleic acid encoding a tissue repair protein, the nucleic acid selected from the group consisting of:

(a) DNA comprising a nucleotide sequence shown in SEQ ID NO:39;

(b) nucleic acids which hybridize to DNA of (a) above under stringent conditions; and

(c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by the DNA of (a) or (b) above.

37. (Amended) An isolated nucleic acid encoding a tissue repair protein, selected from the group consisting of:

(a) DNA comprising a nucleotide sequence shown in SEQ ID NO:3;

(b) nucleic acids which hybridize to DNA of (a) above under stringent conditions; and

(c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by the DNA of (a) or (b) above.

Please add the following new claims:

38. (New) The isolated nucleic acid molecule of claim 1, wherein the nucleic acid comprises at least 75% identity to a nucleic acid sequence shown in SEQ ID NO:39.

39. (New) The isolated nucleic acid molecule of claim 38, wherein the nucleic acid comprises at least 85% identity to a nucleic acid sequence shown in SEQ ID NO:39.

40. (New) The isolated nucleic acid molecule of claim 38, wherein the nucleic acid comprises at least 95% identity to a nucleic acid sequence shown in SEQ ID NO:39.

41. (New) The polypeptide or protein of claim 7, wherein the nucleic acid sequence encodes a protein comprising an amino acid sequence shown in SEQ ID NO:2.

42. (New) The polypeptide or protein of claim 22, wherein the nucleic acid sequence encodes a protein comprising an amino acid sequence shown in SEQ ID NO:4.

Remarks

Claims 1-34 and 36-37 have been amended herein. Claims 38-42 have been added. Thus claims 1-42 are pending.

No new matter has been added by this amendment, nor have any claims been narrowed. The claims were amended solely for the purpose of complying with U.S. claiming conventions.

A new sequence listing is submitted. The sequence listing incorporates the human nucleic acid coding sequence shown in FIG. 7, as SEQ ID NO. 39.

Claims 1, 2, 4, 9-11, 17, 19-22, 28-33, and 36-37 were amended to correct the antecedent basis.

Claims 1-4 and 19-21 were amended to introduce the term "isolated." Support can be found on page 4, lines 12-20.

Claims 2, 4, 8-11, 13-15, 17, 20, and 23-25 and were amended to change the phrase "according to" to "of" in accordance with U.S. claiming conventions.

Claims 3, 4, and 21 were amended to clarify the claim. Support can be found on page 4, lines 38-30.

Claims 5, 6, 12, 17, 18, 26, and 34 were amended to change the "use" claims into method claims. Support can be found as follows:

Claim 5: page 6, line 28-page 7, line 3 and page 8, lines 5-6

Claim 6: page 6, line 28-page 7, line 3

Claim 12: page 8, lines 4-6

Claim 17: page 9, lines 1-2

Claim 18: page 8, lines 19-31

Claim 26: page 8, lines 4-6

Claim 34: page 10, lines 26-29 and page 14, lines 16-27

Claim 7 was amended to correct the spelling of "epitope."

Claims 7 and 22 were amended and to clarify the claims. In addition, claim 22 now depends from claim 7. Support can be found in the specification on page 5, lines 19-23, and in FIG. 7.

Claim 10 was amended to clarify the claim. Support can be found in the specification on page 8, line 1.

Claim 13 was amended to correct the dependency and to clarify the claim. Support can be found in the specification on page 8, lines 12-17.

Claims 15, 16, and 19 were amended to place the verb into an active form.

Claim 24 was amended to clarify the claim. Support can be found in the specification on page 10, lines 1-3.

Claim 27 was amended to clarify the claim. Support can be found in the specification on page 10, lines 5-7 and page 14, line 29 through page 15, line 25.

Claims 28-32 and 36-37 were amended to remove redundant claim language.

Claim 34 was amended to clarify the claim, and to depend from claim 33. Support can be found in the specification on page 10, lines 26-29 and on page 14, lines 16-26.

Claims 36 and 37 were amended to correct the lettering of the subclaims.

Support for new claims 38-40 can be found in the specification on page 5, lines 19-23, and in FIG. 7.

Support for new claim 41 can be found in the specification on page 7, lines 5-9.

Support for new claim 42 can be found in the specification on page 9, lines 19-23.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

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Marked-up Version of Amended Claims
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

1. (Amended) [A] An isolated nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridises to [the] a nucleic acid sequence shown in [of] SEQ ID NO: 1 under high stringency conditions.

2. (Amended) [A] The isolated nucleic acid molecule [according to] of Claim 1 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

3. (Amended) [A] The isolated nucleic acid molecule [according to either prededing claim which is] of claim 1 or claim 2 wherein the nucleic acid molecule is from a mammal [mammalian in origin]

4. (Amended) [A] The isolated nucleic acid molecule [according to] of Claim 3 [which is derived from] wherein the mammal is a human.

5. (Amended) [Use of the nucleic acid of SEQ ID NO:1, fragments or variants thereof, in determining] A method for diagnosing orofacial clefting in a patient, comprising detecting expression of [mRNA] a nucleic acid sequence shown in SEQ ID NO:1, or fragments or variants of the nucleic acid sequence shown in SEQ ID NO:1, in selected target tissues(s) [for diagnosing orofacial clefting].

6. (Amended) [Use of the nucleic acid of SEQ ID NO:1, fragments or derivatives thereof, in determining the presence of DNA mutations] A method for diagnosing orofacial clefting in patients suffering from, or suspected to be suffering from orofacial clefting comprising detecting a mutation in a nucleic acid sequence shown in SEQ ID NO:1, or fragments or variants of the nucleic acid sequence shown in SEQ ID NO:1.

7. (Amended) A polypeptide or a protein comprising an epitope for an antibody, or a protein modified by one or more amino acid modifications and comprising an epitope, or a [fragment] modified or unmodified fragment comprising an [eptitope] epitope for a tissue repair

protein encoded by [SEQ ID NO:2] a nucleic acid sequence having at least 75% identity to a nucleic acid sequence shown in SEQ ID NO:39.

8. (Twice Amended) A delivery vehicle comprising the nucleic acid molecule [according to] of Claim 1 and/or the polypeptide [according to] of Claim 7, which [optionally] is in the form of a suspension.

9. (Amended) [A] The delivery vehicle [according to] of Claim 8, wherein the delivery vehicle [which] is adapted to deliver [said] the nucleic acid molecule or polypeptide to a selected tissue.

10. (Amended) [Antibodies] An antibody raised against the polypeptide [according to] of Claim 7.

11. (Amended) [Antibodies according to] The antibody of Claim 10 [where] wherein the antibody is [are] a monoclonal antibody.

12. (Amended) [Use of antibodies, fragments or derivatives thereof according to Claim 7 in the diagnosis of orofacial clefting] A method for diagnosing orofacial clefting, comprising detecting expression of a protein recognized by the antibody of claim 10.

13. (Amended) A method for detecting the [antibodies according to Claim 7] antibody of claim 10 in a sample, comprising:

labeling a ligand comprising a protein or protein fragment of SEQ ID NO:2 present in the sample;

contacting [with] the sample with the antibody of claim 10, wherein the antibody is immobilised [antibody against a protein or protein fragment of SEQ ID NO:2, which] which results in binding of the immobilised antibody [has bound thereto] to the [a] labelled ligand [comprising a protein or protein fragment of SEQ ID NO:2,]; and

detecting the labelled ligand bound to the immobilised antibody [or labelled ligand bound to antibody] in the sample.

14. (Twice Amended) A method for the treatment of orofacial clefting, comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule [according to] of Claim 1 and/or polypeptide of Claim 7.

15. (Twice Amended) A method for [the treatment of] treating wounds and/or promoting tissue repair, comprising administering to a patient suffering from a wound and/or tissue damage the nucleic acid molecule [according to] of Claim 1 and/or the polypeptide of Claim 7.

16. (Twice Amended) A method of [treatment of] treating wounds and/or promoting tissue repair, comprising administering to a patient suffering from a wound and/or tissue damage a composition comprising the delivery vehicle of Claim 8.

17. (Twice Amended) A pharmaceutical composition comprising the nucleic acid [according to] of Claim 1 and/or [a] the protein [according to] of Claim 7 [for use as a pharmaceutical].

18. (Twice Amended) A method for treating [Use of the nucleic acid according to Claim 1 and/or the protein according to Claim 7 for the manufacture of a medicament for the treatment of] orofacial clefting and/or wound healing and/or tissue repair comprising administering to a patient the nucleic acid of Claim 1 and/or the protein of Claim 7.

19. (Amended) [A] An isolated nucleic acid molecule [which encodes] encoding a tissue repair protein [and comprise] comprising a nucleotide sequence which hybridises to [the] a nucleic acid sequence shown in [of] SEQ ID NO:3 under high stringency conditions.

20. (Amended) [A] The isolated nucleic acid [according to] of Claim 19 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

21. (Twice Amended) [A] The isolated nucleic acid [according to] of Claim 19 [that is murine in origin] wherein the nucleic acid molecule is from a mouse.

22. (Amended) [A] The polypeptide or a protein of claim 7, [comprising an epitope for an antibody or a protein modified by one or more amino acid modifications and comprising an epitope, or a fragment modified or unmodified comprising an eptitope, for a tissue repair protein encoded by SEQ ID NO:4] wherein the nucleic acid sequence comprising at least 75% identity to the nucleic acid sequence shown in SEQ ID NO:39 comprises a nucleic acid sequence shown in SEQ ID NO:3, or a fragment thereof.

23. (Twice Amended) A delivery vehicle comprising the isolated nucleic acid molecule [according to] of Claim 20 and/or the polypeptide [according to] of Claim 22.

24. (Amended) [Antibodies] An antibody raised against the polypeptide [according to] of Claim 22.

25. (Amended) [Antibodies according to] The antibody of Claim 24 [where] wherein the antibody is [are] a monoclonal antibody.

26. (Twice Amended) [Use of antibodies according to Claims 24 in] A method for diagnosing or detecting orofacial clefting comprising detecting expression of a protein recognized by the antibody of claim 24.

27. (Amended) A method of producing a transgenic mammal comprising disrupting a gene, or the effective part [thereof,] of the gene, wherein the gene [encoding] encodes at least one tissue repair protein, and a resulting phenotype is a cleft palate in the transgenic mammal.

28. (Amended) [A] The method of [producing a transgenic mammal according to] Claim 27 wherein the transgenic mammal is a rodent.

29. (Twice Amended) [A] The method of [producing a transgenic mammal according to] Claim 28 wherein the [transgenic mammal] rodent is a mouse.

30. (Twice Amended) [A] The method of [producing the transgenic mammal according to] Claim 27 wherein the gene encoding the tissue repair protein is [the] a nucleic acid molecule comprising a nucleotide sequence which hybridizes to [the] a nucleic acid sequence shown in [of] SEQ ID NO: 3 under high stringency conditions.

31. (Amended) [A] The method of [producing a transgenic mammal according to] Claim 27 wherein the transgenic mammal is a human.

32. (Twice Amended) [A] The method of [producing a transgenic mammal according to] Claim 27 wherein the gene encoding the tissue repair protein is a nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridizes to [the] a nucleic acid sequence shown in [of] SEQ ID NO: 1 under high stringency conditions.

33. (Amended) A reporter gene construct based on [the] a promoter region of a gene, or an effective part thereof encoded by SEQ ID NO:1, or a fragment or variant thereof.

34. (Amended) A method for [Use of a reporter gene construct based on the promoter region of a gene or effective part thereof, encoded by SEQ ID NO:1 in the] detection/screening [of] a pharmaceutical[s] and/or other compound[s], comprising:

contacting the pharmaceutical and/or other compound with the reporter gene construct of claim 33; and

determining a transcriptional response of the reporter gene to the pharmaceutical or other compound wherein the transcriptional response is an indicator of a [and their] potential teratogenic effect[s] of the pharmaceutical or other compound.

36. (Amended) An isolated nucleic acid encoding a tissue repair protein, the nucleic acid [may be] selected from the group consisting of:

(a) DNA [having the] comprising a nucleotide sequence [given herein as] shown in SEQ ID NO: 39[1 (which encodes the protein having the amino acid sequence given herein as SEQ ID NO:2), and which encodes a tissue repair protein];

[(f)] (b) nucleic acids which hybridize to DNA of (a) above [(e g.,] under stringent conditions[) and which encode a tissue repair protein]; and

[(g)] (c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by [a] the DNA of (a) or (b) above.

37. (Amended) An isolated nucleic acid encoding a tissue repair protein, [the nucleic acid may be] selected from the group consisting of:

(a) DNA [having the] comprising a nucleotide sequence [given herein as] shown in SEQ ID NO:3 [(which encodes the protein having the amino acid sequence given herein as SEQ ID NO:4), and which encodes a tissue repair protein];

[(h)] (b) nucleic acids which hybridize to DNA of (a) above [(e.g.,] under stringent conditions[) and which encode a tissue repair protein]; and

[(i)] (c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by [a] the DNA of (a) or (b) above.

38. (New) The isolated nucleic acid molecule of claim 1, wherein the nucleic acid has at least 75% identity to a nucleic acid sequence shown in SEQ ID NO:39.

39. (New) The isolated nucleic acid molecule of claim 38, wherein the nucleic acid has at least 75% identity to a nucleic acid sequence shown in SEQ ID NO:39.

40. (New) The isolated nucleic acid molecule of claim 38, wherein the nucleic acid has at least 95% identity to a nucleic acid sequence shown in SEQ ID NO:39.

41. (New) The polypeptide or protein of claim 7, wherein the nucleic acid sequence encodes a protein comprising an amino acid sequence shown in SEQ ID NO:2.

42. (New) The polypeptide or protein of claim 22, wherein the nucleic acid sequence encodes a protein comprising an amino acid sequence shown in SEQ ID NO:4.

09/869564

Attorney Reference Number 5585-59367
Express Mail Label No. EL828141760US
Date of Deposit: June 29, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Markham *et al.*

Application No.

Filed: Herewith

For: WOUND HEALING AND OROFACIAL CLEFTING

Examiner:

Date: June 29, 2001

Art Unit:

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on June 29, 2001, as Express Mail Label No. EL828141760US, in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

William D. Noonan

William D. Noonan, M.D., Attorney for Applicant

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

Before calculating the filing fee for the present application, please amend the claims as follows:

8. (Amended) A delivery vehicle comprising the nucleic acid molecule according to Claim 1 and/or the polypeptide according to Claim 7, which optionally is in the form of a suspension.

14. (Amended) A method for the treatment of orofacial clefting comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule according to Claim 1 and/or polypeptide of Claim 7.

15. (Amended) A method for the treatment of wounds and/or tissue repair comprising administering to a patient suffering from a wound and/or tissue damage the nucleic acid molecule according to Claim 1 and/or the polypeptide of Claim 7.

16. (Amended) A method of treatment of wounds and/or tissue repair comprising administering to a patient suffering from a wound and/or tissue damage a composition comprising the delivery vehicle of Claim 8.

17. (Amended) A nucleic acid according to Claim 1 and/or a protein according to Claim 7 for use as a pharmaceutical.

18. (Amended) Use of the nucleic acid according to Claim 1 and/or the protein according to Claim 7 for the manufacture of a medicament for the treatment of orofacial clefting and/or wound healing and/or tissue repair.

21. (Amended) A nucleic acid according to Claim 19 that is murine in origin.

23. (Amended) A delivery vehicle comprising the isolated nucleic acid molecule according to Claim 20 and/or the polypeptide according to Claim 22.

26. (Amended) Use of antibodies according to Claim 24 in diagnosing and/or detecting orofacial clefting.

29. (Amended) A method of producing a transgenic mammal according to Claim 28 wherein the transgenic mammal is a mouse.

30. (Amended) A method of producing the transgenic mammal according to Claim 27 wherein the gene encoding the tissue repair protein is the nucleic acid molecule comprising a nucleotide sequence which hybridizes to the nucleic acid of SEQ ID NO: 3 under high stringency conditions.

32. (Amended) A method of producing a transgenic mammal according to Claim 27 wherein the gene encoding the tissue repair protein is a nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridizes to the nucleic acid of SEQ ID NO: 1 under high stringency conditions.

REMARKS

The claims in this application have been amended, solely for the purpose of complying with U.S. claiming conventions.

Respectfully submitted,

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Marked-up Version of Amended Claims
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

Claims

8. (Amended) A delivery vehicle comprising the nucleic acid molecule according to [any of Claims 1-4] Claim 1 and/or the polypeptide according to Claim 7, which optionally is in the form of a suspension.

14. (Amended) A method for the treatment of orofacial clefting comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule according to [any of Claims 1-4] Claim 1 and/or polypeptide of Claim 7.

15. (Amended) A method for the treatment of wounds and/or tissue repair comprising administering to a patient suffering from a wound and/or tissue damage the nucleic acid molecule [molecule] according to [any of Claims 1-4] Claim 1 and/or the polypeptide of Claim 7.

16. (Amended) A method of treatment [according to either Claim 14 or Claim 15 wherein said nucleic acid molecule and/or polypeptide is administered by the incorporation of said nucleic acid molecule into a delivery vehicle according to either of Claims 8 or 9] of wounds and/or tissue repair comprising administering to a patient suffering from a wound and/or tissue damage a composition comprising the delivery vehicle of Claim 8.

17. (Amended) A nucleic acid according to [any of Claims 1-4] Claim 1 and/or a protein according to Claim 7 for use as a pharmaceutical.

18. (Amended) Use of the nucleic acid according to [any of Claims 1-4] Claim 1 and/or [a] the protein according to Claim 7 for the manufacture of a medicament for the treatment of orofacial clefting and/or wound healing and/or tissue repair.

21. (Amended) A nucleic acid according to [either Claim 18 or] Claim 19 that is murine in origin.

23. (Amended) A delivery vehicle comprising the isolated nucleic acid molecule according to [any of Claims 19-21] Claim 20 and/or the polypeptide according to Claim 22.

26. (Amended) Use of antibodies according to [either of Claims 24 or 25] Claim 24 in diagnosing and/or detecting orofacial clefting.

29. (Amended) A method of producing a transgenic mammal according to [either of Claims 27 or 28] Claim 28 wherein the transgenic mammal is a mouse.

30. (Amended) A method of producing [a] the transgenic mammal according to [any of Claims 27-29] Claim 27 wherein the gene encoding the tissue repair protein is the nucleic acid molecule [according to any of Claims 19-21] comprising a nucleotide sequence which hybridizes to the nucleic acid of SEQ ID NO: 3 under high stringency conditions.

32. (Amended) A method of producing a transgenic mammal according to [either of Claims 27 or 31] Claim 27 wherein the gene encoding the tissue repair protein is [the] a nucleic acid molecule [according to any of Claims 1-4] which encodes a tissue repair protein and comprises a nucleotide sequence which hybridizes to the nucleic acid of SEQ ID NO: 1 under high stringency conditions.

Wound Healing and Orofacial Clefting

The present invention relates to the isolation of a nucleic acid molecule and the protein encoded thereby; and the use of these products as therapeutic agents particularly, but not exclusively, in gene therapy and/or tissue repair such as, without limitation enhancing wound and tissue healing and for the treatment of orofacial clefting.

Background to the Invention

Orofacial clefting is the most common human malformation, with an overall incidence of approximately 1 in 600 births. Cleft palate (CP) requires complex management and follow up by multi-disciplinary medical and surgical teams. It has a major impact on both communication and psychological morbidity. Both animal and human studies have shown that cleft palate can be the end result of a number of different aetiological processes. Amongst the teratogenic agents that can cause CP are common drugs (diazepam, sodium valproate, alcohol etc.). Maternal diabetes also confers an increased risk of CP. However, twin studies and familial segregation analyses in a number of different populations have consistently shown that there is a major genetic component to the aetiology of this common developmental abnormality. Despite this, little is known about the specific genetic defects underlying CP. The care of children with clefting is now concentrated in supra-regional centres where the necessary resources can be assembled.

Isolated cleft palate (CPO) is a common human malformation, with a total birth incidence of 1 in 1250 in the West of Scotland [FitzPatrick et al. 1994]. Significant familial clustering [Carter et al. 1982; Shields et al., 1981; Christensen & Fogh-Andersen 1993] and twin studies [Shields et al. 1979] have both suggested that there is a major genetic component in the etiology of CPO. These studies and others [Fogh-Andersen, 1942] have also shown that CPO and cleft lip with or without cleft palate (CL(P)) are genetically distinct subgroups of orofacial clefting. CPO is a

common feature of chromosomal abnormalities, affecting ~15% of all cases of simple autosomal aneuploidy (Schinzel 1994) and is associated with more than 370 different malformation syndromes (Baraitser & Winter, 1997). However, in non-syndromic CL(P) or CPO, relative risk ratio analyses have indicated that there may be a relatively small number of interacting causative loci [FitzPatrick & Farrell, 1993; Christiensson & Mitchell, 1996]. As yet, no disease-causing mutations in non-syndromic CPO have been identified. In the present application we identify for the first time a previously unrecognised gene for cleft palate located at 2q32.

Development of the secondary palate, particularly in mice, has been used as a paradigm in developmental biology, and extensive descriptive studies of the distribution of proteins with putative roles in this process have been published. This knowledge base has led to the extensive use of candidate gene approaches in attempts to unravel the genetic basis of human cleft palate. However, these studies have met with limited success. Purely genetic approaches, in contrast, by the identification of genes causing rare syndromic forms of cleft palate, have yielded greater insights. An example of this is the positional cloning of the gene responsible for Treacher Collins syndrome. Experimental mouse models are another source of valuable information on clefting, for example the transgenic knock outs of Tgfβ3 and Msx1. However, extrapolation from these models by testing for genetic association in human populations has been generally unsuccessful.

There are a number of semi-dominant and recessive mouse models of isolated cleft palate, for example Twirler (Tw) and Dancer (Dc). These provide excellent opportunities to identify potential susceptibility loci for human CP via the positional cloning of the mouse genes. However, none of the loci for these mouse models lies within a region of conserved synteny with the human chromosome 2q32 region a gene from which is the subject of this application.

The problem of identifying susceptibility loci for human malformations has recently been addressed by utilising the common phenomenon of autosomal aneuploidy.

Using the Human Cytogenetics Database, the entire set of information available on post-natally ascertained cases of simple autosomal aneuploidy has been statistically analysed in a manner not previously attempted. This has allowed the identification of specific autosomal regions that are significantly associated with particular malformations. 37 different malformations have been studied, covering a variety of different developmental processes. For cleft palate, 5 different putative loci were identified. The validity of the approach was confirmed by the identification of loci on 4p and 4q, which have previously been suggested to harbour CP susceptibility genes as a result of association or linkage studies. Unexpectedly a major new locus has been identified at 2q32-q33. Interestingly, the penetrance of haploinsufficiency in causing cleft palate at this locus appears to be higher than that of the other four loci. This region has not previously been implicated in the pathogenesis of CP. We have therefore gone on to obtain further independent evidence for the importance of this locus.

From our studies we have identified a human gene which, when dysfunctional, unexpectedly results in cleft palate in man. Replacement of gene function is therefore useful in the treatment of cleft palate and the related cleft lip. There is precedent that disruption of the TGFβ3 gene, for example, leads to the development of cleft palate in animal models. There is also precedent that the use of TGFβ3, for example, is beneficial in the treatment of wounds to achieve enhanced rates of healing. The gene and the protein of the present invention are also therefore useful therapeutically to enhance wound healing. They are also useful clinically to improve tissue repair or regeneration in other clinical contexts, for example in inducing repair of damage to cartilage or boney tissue.

In the present application, we believe we have demonstrated the unexpected existence of an important locus on human chromosome 2q32 causing cleft palate. We have cloned this CP gene and found mutations in a large cohort of patients with cleft palate. Given the problems of day-to-day clinical management of patients with this distressing condition, we also expect that the diagnostic tools described herein

will be rapidly exploited clinically and possibly suggest approaches to prevention strategies for this common malformation.

It is therefore an object of the present invention to provide a tissue repair gene and/or protein for use as a therapeutic agent.

It is a further object of the present invention to provide a tissue repair gene and/or protein for use as a diagnostic agent.

Statements of the Invention

According to a first aspect of the present invention is an isolated nucleic acid encoding a tissue repair protein, the nucleic acid may be selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 (which encodes the protein having the amino acid sequence given herein as SEQ ID NO:2), and which encodes a tissue repair protein;

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode a tissue repair protein ; and

(c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by a DNA of (a) or (b) above.

DNAs of the present invention include those coding for proteins homologous to, and having essentially the same biological properties as, the proteins disclosed herein, and particularly the DNA disclosed herein as SEQ ID NO:1 and encoding the protein given herein SEQ ID NO:2 This definition is intended to encompass natural allelic variations therein. Thus, isolated DNA or cloned genes of the present invention can be of any species of origin, including mouse, rat, rabbit, cat, porcine, and human, but are preferably of mammalian origin. For example, the mouse homologue SEQ ID NO:3 of the human protein encoded by SEQ ID NO:1, differs by only 2 amino acids

in a total of 733 amino acids. Thus. DNAs which hybridize to DNA disclosed herein as SEQ ID NO:1 (or fragments or derivatives thereof which serve as hybridization probes as discussed below) and which code on expression for a protein of the present invention (e.g., a protein according to SEQ ID NO:2), i.e. the tissue repair protein of the present invention associated with orofacial clefting and/or wound healing are to be included in the definition.

Conditions which will permit other DNAs which code on expression for a protein of the present invention to hybridize to the DNA of SEQ ID NO:1 disclosed herein can be determined in accordance with known techniques. For example, hybridization of such sequences may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% Formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to DNA of SEQ ID NO:1 disclosed herein in a standard hybridization assay. See, e.g., J. Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989) (Cold Spring Harbor Laboratory). In general, sequences which code for proteins of the present invention and which hybridize to the DNA of SEQ ID NO:1 disclosed herein will be at least 75% homologous, 85% homologous, and even 95% homologous or more with SEQ ID NO:1. Further, DNAs which code for proteins of the present invention, or DNAs which hybridize to that given as SEQ ID NO:1, but which differ in codon sequence from SEQ ID NO:1 due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the literature. See, e.g., U.S. Patent No. 4,757,006 to Toole et al. at Col. 2, Table 1.

According to a further aspect of the present invention is an isolated nucleic acid encoding a tissue repair protein, the nucleic acid may be selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:3 (which encodes the protein having the amino acid sequence given herein as SEQ ID NO:4), and which encodes a tissue repair protein;

(d) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode a tissue repair protein ; and

(e) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by a DNA of (a) or (b) above.

According a further aspect of the invention there is therefore provided a cloned nucleic acid molecule encoding a tissue repair protein contained in a Yeast Artificial Chromosome species designated as AB 1380 YAC-CP1 and deposited with NCIMB Limited of Aberdeen, Scotland (UK) under accession number NCIMB 41005.

According to a yet further aspect of the invention there is provided a nucleic acid molecule which encodes a tissue repair protein and comprises a nucleotide sequence which hybridises to the nucleic acid of SEQ ID NO:1 under high stringency conditions.

Preferably, hybridisation occurs under stringent conditions such as 1 x SSC, 0.1% SDS at 65 °C.

Preferably, the nucleic acid is mammalian in origin and more preferably is human.

According to a yet further aspect of the invention there is provided use of the nucleic acid of SEQ ID NO:1, fragments and/or variants thereof, in determining expression of mRNA in selected target tissue(s) for diagnosing cleft palate.

According to a yet further aspect of the invention there is provided use of the nucleic acid of SEQ ID NO:1, fragments and/or variants thereof, in determining the presence of mutants in the DNA and thus diagnosing patients suffering from cleft palate.

According to a further aspect of the invention there is provided a polypeptide, or a protein comprising an epitope for an antibody or a protein modified by one or more amino acid modifications and comprising an epitope, or a fragment modified or unmodified comprising an eptitope for a tissue repair protein encoded by SEQ ID NO:2. Ideally the polypeptide is encoded by the nucleic acid molecule of SEQ ID NO:1.

According to a yet further aspect of the invention there is provided a delivery vehicle comprising the isolated nucleic acid molecule or polypeptide of the invention.

Reference herein to the term delivery vehicle is intended to include any vector whether a viral vector or otherwise for example, without limitation, an adenovirus, a retrovirus, a herpesvirus, a plasmid, a phage, a phagemid or a liposome.

Ideally said delivery vehicle is adapted for administration, for example, but without limitation, by suitable formulation into a suspension.

More preferably, said delivery vehicle is adapted to deliver said nucleic acid molecule or polypeptide to selected tissue. Thus the delivery vehicle is provided with means to enable the nucleic acid molecule or polypeptide to be targeted to a specific site. The nature of the means comprises conventional technologies well known to those skilled in the art for example, without limitation, in the instance where the delivery vehicle is a viral vector said viral vector is provided with surface protein adapted to ensure the viral vector binds to and/or penetrates specific target tissues. Thus, in this way, the nucleic acid molecule or peptide, fragments or derivatives thereof of the invention can be used in gene therapy treatments.

According to a yet further aspect of the invention there is provided antibodies raised against the polypeptide, fragment or derivative thereof, of the invention. Ideally the antibodies are monoclonal and more ideally genetically engineered to be humanised. It will be apparent to those skilled in the art that the antibodies of the invention can be used to determine the expression of the polypeptide of the invention in selected target tissue and thus aid in the diagnosis of patients suffering from cleft palate.

According to a yet further aspect of the invention there is provided use of antibodies, fragments or derivatives thereof in diagnosis of orofacial clefting. It will be appreciated that the fragments or derivatives of the antibodies contain the epitope.

According to a yet further aspect of the invention there is provided a method for detecting the antibodies as described above, in a sample, comprising contacting with the sample immobilised antibody against a protein or protein fragment of SEQ ID NO:2, which antibody has bound thereto a labelled ligand comprising a protein or protein fragment of SEQ ID NO:2, and detecting labelled ligand bound to immobilised antibody or labelled ligand bound to antibody in the sample.

According to a yet further aspect of the invention there is provided a method for the treatment of orofacial clefting comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule and/or polypeptide of SEQ ID NO:1 and/or SEQ ID NO:2.

According to a further aspect of the invention there is provided a method for the treatment of wounds comprising administering to a patient suffering from tissue damage the nucleic acid molecule and/or polypeptide of SEQ ID NO:1 and/or SEQ ID NO:2.

Preferably, the nucleic acid molecule and/or polypeptide is administered by the incorporation of said nucleic acid molecule into a delivery vehicle as herein described and ideally the method of treatment involves the use of gene therapy.

According to a yet further aspect of the invention there is the nucleic acid and/or protein,as herein before described for use as a pharmaceutical.

According to a yet further aspect of the invention there is provided use of the nucleic acid and/or protein, fragments or variants thereof of SEQ ID NO:1 and/or SEQ ID NO:2 for the manufacture of a medicament for the treatment of orofacial clefting and/or wound healing and/or tissue repair.

According to a yet further aspect of the invention there is provided an isolated nucleic acid molecule, fragment or variant thereof which encodes a tissue repair protein wherein said isolated nucleic acid molecule has a nucleotide sequence which hybridises to the nucleic acid of SEQ ID NO:3 under high stringency conditions.

Preferably, hybridisation occurs under stringent conditions such as 1 x SSC, 0.1% SDS at 65 °C.

Preferably, the nucleic acid is murine in origin.

According to a further aspect of the invention there is provided a polypeptide, or a protein comprising an epitope for an antibody or a protein modified by one or more amino acid modifications and comprising an epitope, or a fragment modified or unmodified comprising an eptitope for a tissue repair protein encoded by SEQ ID NO:4. Ideally the polypeptide is encoded by the nucleic acid molecule of SEQ ID NO:3.

According to a yet further aspect of the invention there is provided a delivery vehicle comprising the isolated nucleic acid molecule or polypeptide, fragments or derivatives thereof of SEQ ID NO:3 and/or SEQ ID NO:4.

According to a yet further aspect of the invention there is provided antibodies raised against the polypeptide, fragment or derivative thereof of SEQ ID NO:3. Ideally the antibodies are monoclonal.

According to a yet further aspect of the invention there is provided a method of producing a transgenic mammal comprising disrupting a gene, or the effective part thereof, the gene encoding at least one tissue repair protein.

Reference herein to disruption is intended to include complete or partial disruption of expression of the tissue repair protein such that the transgenic animal is unable to express levels of the said protein that are typically found in individuals suffering from cleft palate.

Preferably, the transgenic mammal is a rodent and ideally a mouse and more preferably the gene encoding the tissue repair protein is the nucleic acid molecule or fragment or derivative thereof of SEQ ID NO:3.

Preferably, the transgenic mammal is a human and more preferably the gene encoding the tissue repair protein is the nucleic acid molecule or fragment or derivative thereof of SEQ ID NO:1.

According to a yet further aspect of the invention there is provided a reporter gene construct based on the promoter region of a gene, or effective part thereof, encoded by SEQ ID NO:1.

According to a yet further aspect of the invention there is provided use of a reporter gene construct based on the promoter region of a gene, or effective part thereof, encoded by SEQ ID NO:1 in the detection/screening of pharmaceuticals and/or other compounds and their potential teratogenic effects.

Brief Description of the Figures

Embodiments of the invention will now be described by way of example only with reference to the following figures wherein:

Figures 1 A and 1 B represent chromosome painting of chromosome 2q in patients case 1 and case 2 respectively;

Figures 2 A and B represent fluorescent in situ hybridisation of a YAC probe encoding IGF binding protein 5 in patients case 1 and case 2 respectively;

Figures 3 A and B represent fluorescent in situ hybridisation of YAC's containing the markers D25311 and D2S309 respectively, in patients case 1;

Figure 4 represents fluorescent in situ hybridisation of YAC-CP1 in patient case 1;
Figure 5 represents fluorescent in situ hybridisation of YAC-CP1 in patient case 2;

Figure 6 represents the DNA nucleotide sequence of a human tissue repair gene SEQ ID NO:1;

Figure 7 represents the amino acid sequence for the tissue repair protein SEQ ID NO:2, encoded by SEQ ID NO:1;

Figure 8 represents the DNA nucleotide sequence of a mouse tissue repair gene SEQ ID NO:3;

Figure 9 represents the amino acid sequence for the tissue repair protein SEQ ID NO:4, encoded by SEQ ID NO:3;

Table 1 represents oligonucleotides used in the study; and

Table 2 represents the genetic map of the CP-1 region.

Detailed Description of the Invention

We have tested the novel hypothesis that there is a major locus for cleft palate located at human chromosome 2q32. We have cloned and characterised this cleft palate gene.

It is good clinical practice to perform karyotype analysis on all children with cleft palate. By this approach an 11 year old girl was ascertained with a *de novo* balanced reciprocal translocation between chromosomes 2q32 and 11p14. This patient has a midline posterior cleft of the soft palate, mild learning difficulties and subtle craniofacial dysmorphism (Case 2). A second patient was then studied (Case 1) with a similar clinical phenotype and another de novo balanced reciprocal translocation, this time between chromosomes 2q32 and 7p21. These patients have remarkably consistent clinical features. The existence of 2 different individuals with balanced translocations, possibly involving a common break point at 2q32, strongly supported the conclusion of aneuploidy studies, that an important susceptibility locus for cleft palate exists in this cytogenetic region.

We have therefore taken a molecular cytogenetic route to further analyse these two patients. Initially, we wished to establish that the two break points on 2q32 were indeed located in the same region. A number of experiments using single chromosome painting were performed, which confirmed that both re-arrangements were apparently simple balanced reciprocal translocations and that to a first approximation, they occurred at the same place on chromosome 2q (Figure 1). Next, we tested the possible involvement of a number of potential candidate genes in this region. Yeast artifical chromosomes (YACs) containing these candidates were isolated and each was used for fluorescent in situ hybridization (FISH) analysis. This showed, for example, that the fibronectin and IGF binding protein 5 (IGFBP5) genes are both telomeric to the breakpoints (Figure 2).

We then isolated a total of 70 YACs using genetic markers distributed across the whole 2q32-q33 region. FISH analysis was performed systematically using each of these YACs, in order to position the breakpoints more precisely. Typical results from this study are presented in Figure 3. In support of our initial hypothesis, the FISH analyses confirm that the 2q breakpoints in both patients do indeed lie in the same small interval. We narrowed this region to less than 2 centiMorgans, by virtue of the fact that YACs containing the marker D2S311 are centromeric to both patients' breakpoints, while YACs containing D2S309 are telomeric to the breakpoints, again in both patients (Figure 3 shows Case 1; the data for Case 2 are similar).

On the basis of this information, a YAC contig spanning this interval has been constructed. This has enabled us to resolve a number of uncertainties in current genetic maps of this region. We have placed 23 genetic markers within this small interval and isolated a total of 33 corresponding genomic clones. Our FISH results narrowed the region containing both breakpoints to <600 kb. The YAC clone CP-1 (Fig. 4, 5) crosses both breakpoints. Clearly, this means that we have cloned the novel CP gene. The novel gene is defined by the break points in patients "Case 1" (0213) and "Case 2" (0145) at the point where they occur within YAC-CP1 which has been deposited with the NCIMB Limited under accession number 41005. Cell lines from Case 1 are available in the form of lymphoblastoid cells from ECACC (Porton Down, UK).

The YAC clone spanning both breakpoints was used to screen flow-sorted, chromosome 2 specific cosmid and PAC libraries. A PAC and cosmid contig of the breakpoint region was constructed by a combination of fingerprinting and sequencing. Individual clones were used in further FISH analysis. This approach leads us to the PAC "CP-1" which has also been deposited as above which defines the region harbouring the CP gene. We then took the most direct route to isolating the gene itself, which was sequencing of this PAC clone.

The genomic DNA sequence was used for gene identification (SEQ ID NO:1) by a combination of EST database searching and computational prediction. We screened cDNA libraries to isolate clones corresponding to transcripts in this region. Together with the genomic sequence, these clones provided us directly with information about the intron-exon structure of the gene, and its promoter. In parallel studies, we also isolated the corresponding mouse gene (SEQ ID NO:3) by techniques well known in the art, which will be a prerequisite for transgenic studies.

Availability of the CP gene now allows us to analyse material from patients with cleft palate. We can look for microdeletions at this locus, using FISH analysis of patients with apparently normal karyotypes. We also undertake sequencing of the gene, using cDNA or genomic DNA substrates as appropriate, in these patients. These diagnostic tests are useful clinically in the management of patients with cleft palate and in genetic counselling of them and their families.

In view of evidence implicating a number of known biologically active molecules, both endogenous and exogenous, in the aetiology of CP, it is interesting to examine the influence of such agents on the expression of the newly isolated 2q32 CP gene. This involves the construction of reporter plasmids containing a reporter gene construct of the gene of SEQ ID NO:1 to examine promoter function of the CP gene. Such studies can then be followed up by a more detailed functional analysis of the promoter sequences including deletion mapping and by direct examination of the transcriptional response of the gene to a variety of agents by methods well known in the art. Thus the subject of the present invention is of value to the pharmaceutical industry for the toxicological evaluation of potential new drugs. Analysis of their effects on expression of the CP gene which is the subject of the present invention, will enable prediction of their possible teratogenicity.

As discussed above, using SEQ ID NO:3, we isolated mouse genomic clones from libraries of strain 129 DNA isogenic with the ES cells in use in our laboratories, by techniques known in the art, having confirmed the organisation of the mouse gene,

we performed transgenic knockout experiments using standard approaches. One specific approach involved the use of the IRES-βgeo targetting construct, which as well as eliminating function of the CP gene, allows monitoring of its expression pattern in the embryo by simple staining for β-galactosidase rather than extensive use of in situ hybridization. Such a transgenic approach also facilitated analysis of the effects of recognised teratogens on the expression of this gene in vivo.

Availability of a transgenic model enabled us to undertake other interesting studies. For example, as the mutation in mouse is variable in penetrance, we are in a position to utilise mouse genetic approaches to map modifier loci. These are likely to have homologues in man which could well be of clinical significance.

We have studied two unrelated individuals with strikingly similar clinical features, in whom there are apparently balanced de novo cytogenetic rearrangements involving the same region of chromosome 2q. We now describe molecular cytogenetic analyses that have localised the translocation breakpoint in both cases to a region of some 0.3 Mb between markers D2S311 and D2S115. This suggests that the true location of these breakpoints is 2q32. Independent support for the existence of a novel locus for cleft palate on 2q32 was obtained by a detailed statistical analysis on all cases in the Human Cytogenetics Database of non-mosaic single contiguous autosomal deletions associated with orofacial clefting. This revealed 2q32 to be one of only three chromosomal regions in which haploinsufficiency is highly significantly associated with isolated cleft palate. In combination, our data provided strong evidence for the existence at 2q32 of a gene that is critical to the development of the secondary palate. The close proximity of the two chromosomal breakpoints also made the positional cloning of this gene a realistic possibility.

Subjects and Methods

Case 1: Case 1, the fourth child of healthy, non-consanguineous parents, was delivered at 38 weeks' gestation, weighing 2.95kg. Cleft palate was noted at birth. Delayed motor development was apparent at four months and the patient did not

walk until two years. Particular problems were noted with the acquisition of language skills. She underwent repair of her cleft palate at eighteen months of age and required pharyngoplasty at eleven years. Her hearing was normal. She had a prominent nasal bridge, a small mouth and long, slender fingers. Her growth has been satisfactory, and her height has always been on or above the 50th centile. Her weight was below the 10th centile until the age of five but at the age of ten was on the 75th centile. Her head circumference was on the 50th centile. She has moderate learning disability. Blood chromosome analysis revealed an apparently balanced reciprocal translocation with the karyotype 46XX, t(2;7)(q33;p21). Parental karyotypes were normal. FISH analysis showed no deletion of 22q11.22.

Case 2: Case 2 was a female delivered at term after an uneventful pregnancy. At birth she was noted to have cleft palate and minor facial dysmorphism. In addition to repair of her palate, she had required surgical correction of a convergent squint. On examination at the age of 8 years, she has fair hair and skin, a long, narrow face with apparent hypotelorism, a prominent nasal bridge and pinched appearance above the nares, and a small mouth and jaw. She had abnormal dermatoglyphics with a reduced ridge count. She was of slender build, with height between the 75th and 90th centiles and weight on the 10th centile. She had mild global developmental delay, particularly in language skills, and was one year behind her peers in a mainstream school. Chromosome analysis revealed an apparently balanced reciprocal translocation with the karyotype 46,XX,t(2;11)(q33;p14). Parental karyotypes were normal. FISH analysis showed no deletion of 22q11.22.

Molecular cytogenetic analysis

Our initial working map across the 2q breakpoint region (Dib et al. 1996) and a modified physical map, based on the consensus map of Collins et al. (1996), adjusted to reflect data that have emerged from our FISH studies, are presented (Table 2). In addition to YACs containing this set of genetic markers, we also isolated YAC clones containing a number of genes that were known to be located in this region but

had not been finely mapped. These were selected on the basis of their potential involvement in the etiology of cleft palate (Table I).

Statistical analysis of chromosomal deletions

By analysing all cases of single, contiguous, non-mosaic autosomal deletions stored in the Human Cytogenetics Database (HCDB) (Schinzel, 1994) three chromosomal regions (2q32, 4p16-13, 4q31-35) were identified where monosomy is non-randomly associated with CPO. However, HCDB searches alone do not differentiate cleft palate in the context of CL(P) cases from those cases that have CPO. As these are etiologically distinct subgroups of orofacial clefting and might be expected to have different causative genetic loci, the precise significance of these three chromosomal loci must necessarily be unclear. For a better insight into the phenotypes associated with deletion of these regions, the original case reports of all cases were obtained and reviewed. A statistical reanalysis was then performed as before but confining the analysis to confirmed CPO cases. Briefly, the distribution of deletions of regions including bands on chromosomes 4 or 2q in CPO patients was determined. The observed number of CPO-associated deletions of each band was compared with the expected number, calculated from the distribution of all band deletions on chromosomes 4 and 2q in HCDB. The number of deletions of any band was taken to follow a Poisson distribution, since this number is usually small. Confidence limits for the observed number of deletions and the significance of any deviation from expectation were calculated as described by Vasarhelyi and Friedman (1989).

Results

Patient phenotypes

Initial clues to the existence of a CPO locus on 2q32-33 came through the identification of a patient (Case 2) with a de novo balanced reciprocal translocation t(2;11)(q33;p14). A second patient (Case 1) was identified, again with CPO and a de

novo translocation [t(2;7)(q33;p21)] involving the same cytogenetic band on chromosome 2. Both patients had strikingly similar clinical appearances.

FISH Analysis

Initial chromosome painting studies confirmed that the sizes of the translocated 2q fragments were approximately the same in both patients (Fig. 1A, 1B). To attempt to establish whether the breakpoints in Cases 1 and 2 had occurred within the same region of chromosome 2q32, single locus FISH analysis was then conducted, using a large collection of YACs containing markers mapping within the 2q32-33 region. The results of this FISH study are summarised in Table 1. YACs containing the candidate genes FN1 (fibronectin) (Iamaroon 1996), IGFBP5 (Ferguson et al. 1992), IGFBP2 (not shown) and IHH (Indian Hedgehog) (Leek et al 1997, not shown) were all found to map distal (telomeric) to both chromosome 2 breakpoints.

Markers flanking both patients' 2q breakpoints were next identified by FISH (Fig. 3A, B). The signal generated by YACs containing D2S311 is present on both the normal and derivative copies of chromosome 2, in both Cases 1 and 2. Thus D2S311 is proximal (centromeric) to both breakpoints; this is clearly shown through simultaneous hybridization to the centromeric probes D2Z1 and either D7Z1 or D11Z1. In contrast, FISH with YACs containing D2S309, or D2S116 gave signals lying distal to the breakpoint in both individuals. This suggested that both breakpoints lie within a common region of some 2.5 Mb of 2q32. To reduce this interval further, YACs 14HA2 (containing D2S2189), 26IF5 (containing D2S1384/D2S307/ CTLA4/D2S105/D2S72) and 11GG8 (containing D2S115) were analysed. All were found to map distal to the 2q32 breakpoint in both patients. In this way, by systematic FISH analysis, the breakpoint region in both these patients with cleft palate has been localized to an interval, which may be as small as 0.3 Mb, according to current maps (Collins et al. 1996).

We suggest that a previously unrecognised locus causing cleft palate resides in chromosome region 2q32. Our suggestion relies on the integration of clinical, cytogenetic, molecular and statistical data. Two unrelated children had strikingly similar clinical features, each having a de novo cytogenetic rearrangement apparently involving the same band on chromosome 2q. Both girls had cleft palate. They also both had mild learning difficulties and a strikingly similar facial appearance. While their facial dysmorphisms are subtle, these and other clinical features were reminiscent of those seen in velocardiofacial syndrome (VCFS, OMIM 192430). It would appear, however, that these girls do not have VCFS, as neither of them has a cardiac malformation, nor the microdeletion of 22q11.22 seen in most cases of VCFS (Scrambler et al., 1992). The existence of non-22q-deleted phenocopies of VCFS is well recognized (Daw et al., 1996), so that it is possible that a 2q32 locus accounts for a proportion of such cases.

We isolated markers for this region of chromosome 2q, which allowed us to isolate a large number of genomic reagents for precise delineation of the 2q breakpoints in both cases. The 30 separate YACs that have been isolated provide good representation of this 2.5 Mb region.

The initial G-banded cytogenetic studies performed on both patients indicated the existence of a common breakpoint near 2q33. However, our single locus FISH studies collectively strongly suggest that the breakpoint in both Case 1 and Case 2 lies in 2q32. This discrepancy may result from the known bias in reporting of breakpoints in favor of Giemsa-pale bands, or it may be that the true breakpoint is at the 2q32-2q33 band junction. More importantly, both breakpoints mapped to a very small chromosomal region between D2S311 and D2S115, strongly suggesting that the same single gene is disrupted in both patients. These flanking markers are estimated to map at 207.169 Mb and 207.462 Mb respectively, in the current version of the Location Database LDB (Collins et al. 1996; ttp://cedar.genetics.soton.ac.uk/public html/). The cleft palate gene was thus localized by our studies to a region as small as 0.3 Mb. Two additional genetic markers (D2S374 and D2S1413)

have been mapped into this interval. A genomic clone, YAC-CP1, crosses both patients' breakpoints and represents the cloning of this cleft palate gene. Proof that 2q32 contains a CPO-causative genetic locus has been provided by the cloning of a gene whose function is disrupted by both breakpoints in these patients and the demonstration of mutations within this gene in cytogenetically normal individuals with CPO.

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Amended Claims

1. Use of the nucleic acid of SEQ ID NO:1 or SEQ ID NO:3, fragments or variants thereof, in determining expression of mRNA in selected target tissue(s) for diagnosing orofacial clefting.

2. Use of the nucleic acid of SEQ ID NO:1 or SEQ ID NO:3, fragments or derivatives thereof, in determining the presence of DNA mutations in patients suffering from, or suspected to be suffering from orofacial clefting.

3. A polypeptide or a protein comprising an epitope for an antibody or a protein modified by one or more amino acid modifications and comprising an epitope, or a fragment modified or unmodified comprising an eptitope for a tissue repair protein encoded by SEQ ID NO:2 or SEQ ID NO:4, for use in diagnosing orofacial clefting.

4. A delivery vehicle comprising the nucleic acid molecule as defined in either claim 1 or 2 and/or a polypeptide as defined in claim 3, which optionally is in the form of a suspension.

5. A delivery vehicle according to Claim 4 which is adapted to deliver said nucleic acid molecule or polypeptide to a selected tissue.

6. Antibodies against the polypeptide according to Claim 3.

7. Antibodies according to Claim 6 which are monoclonal.

8. Use of antibodies, fragments or derivatives thereof according to either Claim 6 or 7 in the diagnosis of orofacial clefting.

9. A method for detecting the antibodies according to either Claim 6 or 7 in a sample, comprising contacting with the sample immobilised antibody against a protein or protein fragment of SEQ ID NO:2 or SEQ ID NO:4, which antibody has bound thereto a labelled ligand comprising a protein or protein fragment of SEQ ID NO:2 or SEQ ID NO:4, and detecting labelled ligand bound to immobilised antibody or labelled ligand bound to antibody in the sample.

10. A method for the treatment of orofacial clefting comprising administering to a patient suffering from orofacial clefting the nucleic acid molecule as defined in either claim 1 or 2 and/or the polypeptide as defined in claim 3.

11. A method for the treatment of wounds and/or tissue repair comprising administering to a patient suffering from a wound and/or tissue damage the nucleic acid molecule molecule as defined in either claim 1 or 2 and/or the polypeptide as defined in claim 3.

12. A method of treatment according to either Claim 10 or Claim 11 wherein said nucleic acid molecule and/or polypeptide is administered by the incorporation of said nucleic acid molecule into a delivery vehicle according to either of Claims 4 or 5.

13. A nucleic acid as defined in either claim 1 or 2 and/or the polypeptide as defined in claim 3 for use as a pharmaceutical.

14. Use of the nucleic acid as defined in either claim 1 or 2 and/or the polypeptide as defined in claim 3 for the manufacture of a medicament for the treatment of orofacial clefting and/or wound healing and/or tissue repair.

15. A method of producing a transgenic non-human mammal comprising disrupting a gene, or the effective part thereof, the gene encoding at least one tissue repair protein and comprising the nucleic acid sequence as set forth in SEQ ID NO:3.

16. A method according to Claim 15 wherein the transgenic non-human mammal is a rodent.

17. A method according to either of Claims 15 or 16 wherein the transgenic non-human mammal is a mouse.

18. A reporter gene construct based on the promoter region of a gene, or effective part thereof encoded by SEQ ID NO:1 or SEQ ID NO:3 or fragment or variant thereof.

19. Use of a reporter gene construct based on the promoter region of a gene or effective part thereof, encoded by SEQ ID NO:1 or SEQ ID NO:3 in the detection/screening of pharmaceuticals and/or other compounds and their potential teratogenic effects.

20. A cloned nucleic acid molecule encoding a tissue repair protein contained in a Yeast Artificial Chromosome species designated as AB 1380 YAC-CP1 and deposited with NCIMB Limited of Aberdeen, Scotland (UK) under accession number NCIMB 41005.

21. An isolated nucleic acid encoding a tissue repair protein, the nucleic acid may be selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 (which encodes the protein having the amino acid sequence given herein as SEQ ID NO:2), and which encodes a tissue repair protein;

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode a tissue repair protein ; and

(c) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by a DNA of (a) or (b) above.

22. An isolated nucleic acid encoding a tissue repair protein, the nucleic acid may be selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:3 (which encodes the protein having the amino acid sequence given herein as SEQ ID NO:4), and which encodes a tissue repair protein;

(d) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode a tissue repair protein ; and

(e) nucleic acids which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code, and which encode a tissue repair protein encoded by a DNA of (a) or (b) above.

23. A pharmaceutical formulation comprising the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3 fragments or variants or products thereof, and a physiologically acceptable excipient, diluent or carrier.

P32039wo.amendedclaims

FIGURE 1A

FIGURE 1B

FIGURE 2A

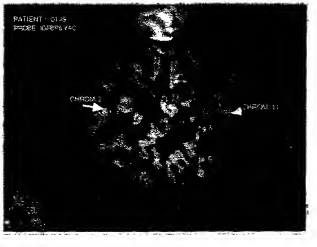

FIGURE 2B

FIGURE 3A

FIGURE 3B

Figure 4

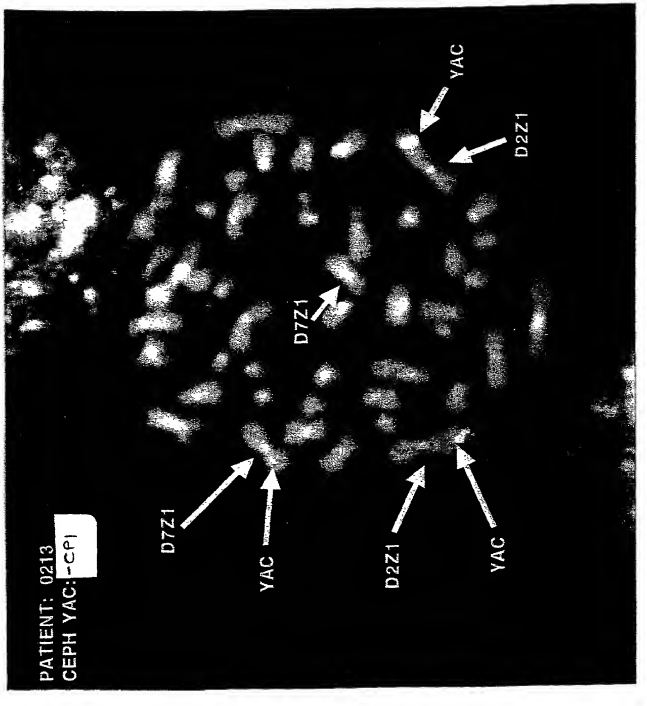

Figure 5

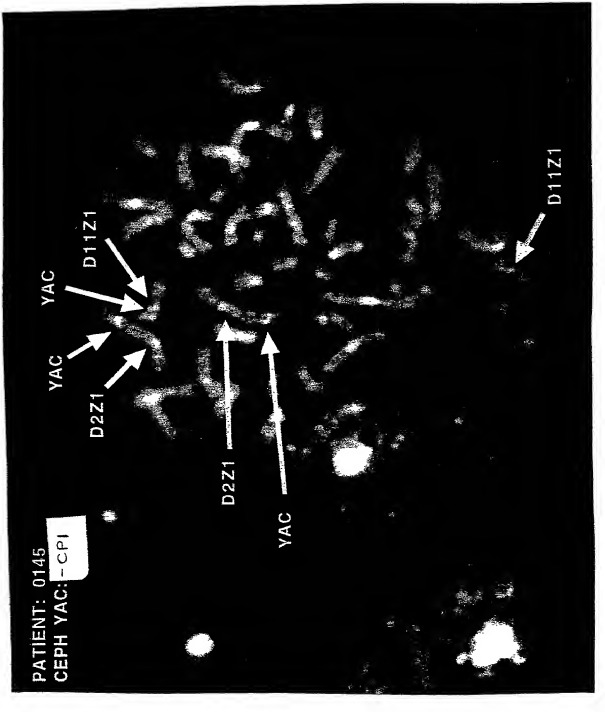

FIGURE 6 SEQ ID NO:1 (Human cDNA)

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 63 actttgtccccgagtcggagcagcatggagcggcggagccgagagcccgtctgcgggac
                                M  E  R  R  S  E  S  P  C  L  R  D
123 agccccgaccggcggagcggcagcccggacgtcaaggggcctccccagtgaaggtggcc
     S  P  D  R  R  S  G  S  P  D  V  K  G  P  P  P  V  K  V  A
183 cggctggagcagaacggcagccccatgggagcccgcggaggagccccaacggcgccgtggcc
     R  L  E  Q  N  G  S  P  M  G  A  R  G  R  P  N  G  A  V  A
243 aaggccgtgggaggtttgatgattcctgtctttgtgtcgtggagcagttggacggctct
     K  A  V  G  G  L  M  I  P  V  F  C  V  V  E  Q  L  D  G  S
303 cttgaatatgacaacagagaagaacacgccgagtttgtcctggtgcggaaagatgtgctt
     L  E  Y  D  N  R  E  E  H  A  E  F  V  L  V  R  K  D  V  L
363 tttagccagctggtggagactgcgctcctggccctggggtattctcacagctctgcggcc
     F  S  Q  L  V  E  T  A  L  L  A  L  G  Y  S  H  S  S  A  A
423 caggcccaaggaataatcaagctgggaaggtggaacctctccccctcagttatgtgaca
     Q  A  Q  G  I  I  K  L  G  R  W  N  P  L  P  L  S  Y  V  T
483 gatgcacccgacgcgacagtggccgcatgctacaagatgtctatcatgttgtgacgttg
     D  A  P  D  A  T  V  A  D  M  L  Q  D  V  Y  H  V  V  T  L
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     A  T  V  R  N  A  L  K  E  L  L  K  E  M  N  Q  S  T  L  A
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     K  E  C  P  L  S  Q  S  M  I  S  S  I  V  N  S  T  Y  Y  A
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     N  V  S  A  T  K  C  Q  E  F  G  R  W  Y  K  K  Y  K  K  I
783 aaagtggaaagagtggaacgagaaaacctttcagactattgtgttctgggccagcgtcca
     K  V  E  R  V  E  R  E  N  L  S  D  Y  C  V  L  G  Q  R  P
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     M  H  L  P  N  M  N  Q  L  A  S  L  G  K  T  N  E  Q  S  P
903 cacagccaaattcaccacagtactccaatccgaaaccaagtgcccgcattacagcccatc
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963 atgagccctggttctttctttcccccagcttagtccacaacttgtaaggcaacaaatagcc
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     Q  A  I  N  Q  Q  F  L  N  H  P  P  I  P  R  A  V  K  P  E
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1263 ttgttgtccgagattctgcgtaaggaagaagaccctcggacagcctctcagtctctttcta
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1323 gtaaacctgagggccatgcagaatttcctcaatctgccagaagtggagcgagatcgcatc
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```

```
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     T  L  W  E  N  L  C  T  I  R  R  F  L  N  L  P  Q  H  E  R
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     D  L  P  K  H  T  I  I  K  F  F  Q  N  Q  R  Y  H  V  K  H
2103 cacgggaagctgaaagagcacctgggctccgcggtggacgtggctgaatataaggacgag
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     E  L  L  T  E  S  E  E  N  D  S  E  E  G  S  E  E  M  Y  K
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2283 agataatgtgaacttctactaggcaaagcaatacatcggtccaaggatttctgctttca
     R  *
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2403 tatttttgtctttttatgtctgtttcgttttcttacccttttggacatttctttgttgc

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2703 tatatatcctatatttacatatatatattaaaattgcatgggacagagactttgcaatc

2763 cgaaagaatagactgtgaaatgagtcctaaagaaaagacttgtttatgtattaaaaaaa

2823 ccacttcacagtgagtcgctttggcttttgataaactgcggcctgctctcagggtgggg

2883 tgactatttttgaattcctatttatttttgtgtttgtccctgatttttttttttaattc

2943 tatggcttcctatctggcagcttaatgggtaatttttgaggtatgtatttaacaaaataa

3003 acgacactgccgaaaaaaaaaaaagcgaagtgaaaacaatcagggcacattaaaatgata

3063 caagtcaaataaatctaaagacacaatgcacacttaaaatgactcaataaaatgacttg

3123 ctacgttccgttattcaatttgtcattactgtagtgaacagatgcatttctgtggaattc

3183 caaataagtaaaactgaaattcagtgcagagaaaactttgtccactagtgcaagtcttga

3243 tcaaatgacattttgacattggacatatggaattcatagtatgagccacattttgttgtg

3303 aaatttatttacctgcttgtggcttcaaatctgaaaattaataagcctgctcgtttaaaa
```

3363 gttgtttgttgttgctgttttttgtcttttgttttttactagaaaatagttcagtgta

3423 atattaagttagaaaagaagttgctgcccagttaaaggggctccctctcaaataaatctc

3483 catcctccctctcccaaaagacatttctgatttctgcttcactttgggcttcctcttct

3543 tcgtacacattccatctacctaatcaaacattttcagtccctgatctctcctgtcccttt

3603 tcctgggatgacagccctaacaagaactgtttttgaatcgttgtgcagctccaggcaata

3663 gagtatgtgaagcgatttcagtagaatcacttactcatcctaaaagaaaacattatccca

3723 gttacctacatcgcaattaccttatgtaaagcagaactaatgctgactggatgtttaatg

3783 ggatgagcattaaagctgcaatctactatagtactccagatctctttcggcttcctatga

3843 gaaacaccagaagcattactttccacttctacttacagtaattgcaagaggagacctcac

3903 attcaggactggcctagtgaacgtaatccatgctttaaactggccattaaacagtcccac

3963 atggttggatttttttttttttttgagttgtgctttcacaaaaccttgtcaaagacctc

4023 atgcaatatcactttgaaagttattttctgtttactacacaaacattgtaatataactgt

4083 taatactatttatatatttgaaaggtataaaaggtaggagttaaaaaaaaaacctctatg

4143 tgtagatattaactcagaacttacaatatacagggagaagacatgttgcaatacaagcta

4203 attctagctgctcagtaacctctggagttttaaagggacattttcctgtacttttcaa

4263 ataatgatgtttaaaaattatcttgacataagcgtcatataccttgcaaaaggatggtt

4323 gtttgcagttagccctggccccatccttcctatttctgtagtatgctgcagctttaatca

4383 gaaagtccatggttgctgcttcctgatctccgagttactctttccaaattgtcttcttac

4443 actgttgctgaaggtcactctgtacacgtaatggaaactgattttgccaagctcttacaa

4503 ggtggttcatctatcgatggcatccgcatttggtatcttttacacttcaaccaaaaattt

4563 attaggtatttttcaatgctaagtcttgccttttatttttttaatttcactgccaagtttg

4623 cagtggttctaagtgaatctgtgggcattttagcctgtggtcttgccagatctttgcgaa

4683 ttacaatgcatatatgtctatttattcaatatctgtcatataatatctatttggaagaag

4743 aaactttcttcttgtagtgcctcttgacaaagcacaatttcccgccttttttttttttttt

4803 gtgaaatgaaaaaaacaaattgtgttttattgcggtatcaacaatgtgaataaggattaa

4863 catattgtaaatgttctttttttccatgtaaatcaactatctttgttatcactaagtgata

4923 attaatttttaacttatgtgcattgttaggctgttagaatttttttggttgttaaaataaa

4983 cgcattcaataaatatg 4999

FIGURE 7 SEQ ID NO:2 (Human amino acid sequence)

```
87 atggagcggcggagcgagagcccgtgtctgcgggacagccccgac
    M  E  R  R  S  E  S  P  C  L  R  D  S  P  D
132 cggcggagcggcagcccggacgtcaaggggcctccccagtgaag
    R  R  S  G  S  P  D  V  K  G  P  P  P  V  K
177 gtggccccggctggagcagaacggcagccccatgggagcccgcggg
    V  A  R  L  E  Q  N  G  S  P  M  G  A  R  G
222 aggcccaacggcgccgtggccaaggccgtgggaggtttgatgatt
    R  P  N  G  A  V  A  K  A  V  G  G  L  M  I
267 cctgtctttgtgtcgtggagcagttggacggctctcttgaatat
    P  V  F  C  V  V  E  Q  L  D  G  S  L  E  Y
312 gacaacagagaagaacacgccgagtttgtcctggtgcggaaagat
    D  N  R  E  E  H  A  E  F  V  L  V  R  K  D
357 gtgctttttagccagctggtggagactgcgctcctggccctgggg
    V  L  F  S  Q  L  V  E  T  A  L  L  A  L  G
402 tattctcacagctctgcggccccaggcccaaggaataatcaagctg
    Y  S  H  S  S  A  A  Q  A  Q  Q  G  I  I  K  L
447 ggaaggtggaacccctctcccccctcagttatgtgacagatgcaccc
    G  R  W  N  P  L  P  L  S  Y  V  T  D  A  P
492 gacgcgacagtggccgacatgctacaagatgtctatcatgttgtg
    D  A  T  V  A  D  M  L  Q  D  V  Y  H  V  V
537 acgttgaaaatccaattacaaagttgttcaaagttggaagacttg
    T  L  K  I  Q  L  Q  S  C  S  K  L  E  D  L
582 cctgcggagcagtggaaccatgccacagtccgcaatgccttaaag
    P  A  E  Q  W  N  H  A  T  V  R  N  A  L  K
627 gaactgctcaaagagatgaaccagagcacattagccaaagaatgc
    E  L  L  K  E  M  N  Q  S  T  L  A  K  E  C
672 cctctctcccagagtatgatttcatccattgtaaatagcacatat
    P  L  S  Q  S  M  I  S  S  I  V  N  S  T  Y
717 tatgccaatgtgtcagcaaccaagtgccaggagtttgggagatgg
    Y  A  N  V  S  A  T  K  C  Q  E  F  G  R  W
762 tataaaaagtacaagaagattaaagtggaaagagtggaacgagaa
    Y  K  K  Y  K  K  I  K  V  E  R  V  E  R  E
807 aacctttcagactattgtgttctgggccagcgtccaatgcattta
    N  L  S  D  Y  C  V  L  G  Q  R  P  M  H  L
852 ccaaatatgaaccagctggcatcccgggggaaaaccaacgaacag
    P  N  M  N  Q  L  A  S  L  G  K  T  N  E  Q
897 tctcctcacagccaaattcaccacagtactccaatccgaaaccaa
    S  P  H  S  Q  I  H  H  S  T  P  I  R  N  Q
942 gtgcccgcattacagcccatcatgagccctggttcttctttctccc
    V  P  A  L  Q  P  I  M  S  P  G  L  L  S  P
987 cagcttagtccacaacttgtaaggcaacaaatagccatggcccat
    Q  L  S  P  Q  L  V  R  Q  Q  I  A  M  A  H
1032 ctgataaacccaacagattgccgttagccggctcctggctcaccag
    L  I  N  Q  Q  I  A  V  S  R  L  L  A  H  Q
1077 catcctcaagccatcaaccagcagttcctgaaccatccaccccatc
    H  P  Q  A  I  N  Q  Q  F  L  N  H  P  P  I
1122 cccagagcagttaagccagagccaaccaactcttccgtggaagtc
    P  R  A  V  K  P  E  P  T  N  S  S  V  E  V
1167 tctccagatatctaccagcaagtcagagatgagctgaagagggcc
    S  P  D  I  Y  Q  Q  V  R  D  E  L  K  R  A
1212 agtgtgtcccaagctgtctttgcaagagtggcattcaaccgcaca
    S  V  S  Q  A  V  F  A  R  V  A  F  N  R  T
1257 cagggattgttgtctgagattctgcgtaaggaagaagaccctcgg
    Q  G  L  L  S  E  I  L  R  K  E  E  D  P  R
1302 acagcctctcagtctcttctagtaaacctgagggccatgcagaat
    T  A  S  Q  S  L  L  V  N  L  R  A  M  Q  N
```

```
1347 ttcctcaatctgccagaagtggagcgagatcgcatctaccaggat
     F  L  N  L  P  E  V  E  R  D  R  I  Y  Q  D
1392 gagagggagcggagcatgaatcccaatgtgagcatggtctcctcg
     E  R  E  R  S  M  N  P  N  V  S  M  V  S  S
1437 gcctccagcagtcccagctcctcccgaaccccctcaggccaaaacc
     A  S  S  S  P  S  S  S  R  T  P  Q  A  K  T
1482 tcgacaccgacaacagacctccctattaaggtggacggcgccaac
     S  T  P  T  T  D  L  P  I  K  V  D  G  A  N
1527 atcaacatcacagctgccatttatgacgagatccaacaggagatg
     I  N  I  T  A  A  I  Y  D  E  I  Q  Q  E  M
1572 aaaagggccaaggtgtctcaagccctgtttgccaaagtggctgca
     K  R  A  K  V  S  Q  A  L  F  A  K  V  A  A
1617 aataaaagccagggctggctgtgtgaactgctccgctggaaggag
     N  K  S  Q  G  W  L  C  E  L  L  R  W  K  E
1662 aacccaagcccagaaaaccgcaccctctgggaaaacctctgtacc
     N  P  S  P  E  N  R  T  L  W  E  N  L  C  T
1707 atccgtcgcttcctgaaccttccccagcatgagagggatgtcatc
     I  R  R  F  L  N  L  P  Q  H  E  R  D  V  I
1752 tatgaggaggagtcaaggcatcaccacagcgaacgcatgcaacac
     Y  E  E  E  S  R  H  H  H  S  E  R  M  Q  H
1797 gtggtccagcttccccctgagccggtgcaggtacttcatagacag
     V  V  Q  L  P  P  E  P  V  Q  V  L  H  R  Q
1842 cagtctcagccagccaaggagagttcccctcccagagaagaagcg
     Q  S  Q  P  A  K  E  S  S  P  P  R  E  E  A
1887 cctcccccacctcctccgactgaagacagttgtgccaaaaagccc
     P  P  P  P  P  P  T  E  D  S  C  A  K  K  P
1932 cggtctcgcacaaagatctccttagaagccctggggatcctccaa
     R  S  R  T  K  I  S  L  E  A  L  G  I  L  Q
1977 agctttattcatgatgtaggcctgtacccagaccaggaagccatc
     S  F  I  H  D  V  G  L  Y  P  D  Q  E  A  I
2022 cacactctttcggctcagctggatctccccaaacacaccatcatc
     H  T  L  S  A  Q  L  D  L  P  K  H  T  I  I
2067 aagttcttccagaaccagcggtaccacgtgaagcaccacgggaag
     K  F  F  Q  N  Q  R  Y  H  V  K  H  H  G  K
2112 ctgaaagagcacctgggctccgcggtggacgtggctgaatataag
     L  K  E  H  L  G  S  A  V  D  V  A  E  Y  K
2157 gacgaggagctgctgaccgagtcagaggagaacgacagcgaggaa
     D  E  E  L  L  T  E  S  E  E  N  D  S  E  E
2202 ggctccgaggagatgtacaaagtggaggctgaggaggaaaatgct
     G  S  E  E  M  Y  K  V  E  A  E  E  E  N  A
2247 gacaaaagcaaggcagcacctgccgaaattgaccagagataa 2288
     D  K  S  K  A  A  P  A  E  I  D  Q  R  *
```

FIGURE 8 SEQ ID NO 3 (Mouse cDNA)

```
  1 atggagcggcggagcgagagcccgtgtcttcgggacagccccgac
    M  E  R  R  S  E  S  P  C  L  R  D  S  P  D
 46 cgaagaagcggcagcccygacgtcaaggggcctcccccggtgaag
    R  R  S  G  S  P  D  V  K  G  P  P  P  V  K
 91 gtggcccggctggagcagaacggcagccccatgggagcccgcggg
    V  A  R  L  E  Q  N  G  S  P  M  G  A  R  G
136 aggcccaacggcgccgtggccaaggccgtgggaggtttgatgatt
    R  P  N  G  A  V  A  K  A  V  G  G  L  M  I
181 ccagttttctgtgtggtggagcagttggatggctctcttgaatac
    P  V  F  C  V  V  E  Q  L  D  G  S  L  E  Y
226 gacaaccgagaagagcacgctgagttcgtcttggtgcggaaagat
    D  N  R  E  E  H  A  E  F  V  L  V  R  K  D
271 gtgcttttagccagctggtggagaccgcgctcctggccctgggg
    V  L  F  S  Q  L  V  E  T  A  L  L  A  L  G
316 tattcccacagctctgcagcgcaggcccaaggaataatcaagcta
    Y  S  H  S  S  A  A  Q  A  Q  G  I  I  K  L
361 gggaggtggaacccctcccctcagttatgtgacagacgcccct
    G  R  W  N  P  L  P  L  S  Y  V  T  D  A  P
406 gatgcgactgtggccgacatgctgcaagatgtctatcacgttgtg
    D  A  T  V  A  D  M  L  Q  D  V  Y  H  V  V
451 acgctgaagatccaattacaaagttgttcaaagttggaagacttg
    T  L  K  I  Q  L  Q  S  C  S  K  L  E  D  L
496 cctgcggagcaatggaaccacgccaccgtccgcaatgccttaaag
    P  A  E  Q  W  N  H  A  T  V  R  N  A  L  K
541 gaactgctcaaagaaatgaaccagagcacattagccaaagaatgc
    E  L  L  K  E  M  N  Q  S  T  L  A  K  E  C
586 cctctctcccagagtatgatttcatccattgtaaatagcacatac
    P  L  S  Q  S  M  I  S  S  I  V  N  S  T  Y
631 tatgccaatgtgtcagcaaccaagtgccaggagtttgggagatgg
    Y  A  N  V  S  A  T  K  C  Q  E  F  G  R  W
676 tacaaaaagtataagaagataaaagtggaaagagtggagcgagag
    Y  K  K  Y  K  K  I  K  V  E  R  V  E  R  E
721 aacctttcagactattgtgttctgggccagcggccaatgcattta
    N  L  S  D  Y  C  V  L  G  Q  R  P  M  H  L
766 ccaaatatgaaccagctggcatccctgggcaaaaccaacgaacag
    P  N  M  N  Q  L  A  S  L  G  K  T  N  E  Q
811 tctcctcatagccaaatccaccacagtactccaatccgaaaccaa
    S  P  H  S  Q  I  H  H  S  T  P  I  R  N  Q
856 gtgcccgcactccagcccatcatgagccctggtcttctctcaccg
    V  P  A  L  Q  P  I  M  S  P  G  L  L  S  P
901 cagctcagtcctcagcttgtcaggcagcaaatagccatggcccat
    Q  L  S  P  Q  L  V  R  Q  Q  I  A  M  A  H
946 ctgataaaccaacagatagccgttagccgactcctggctcaccag
    L  I  N  Q  Q  I  A  V  S  R  L  L  A  H  Q
991 catcctcaagccatcaaccagcagttcttgaaccacccacccatt
    H  P  Q  A  I  N  Q  Q  F  L  N  H  P  P  I
```

```
1036 cccagagcagttaagccagagccaacaaactcctctgtggaagtc
     P  R  A  V  K  P  E  P  T  N  S  S  V  E  V
1081 tctcctgatatctaccagcaagttagagatgagttgaagagggct
     S  P  D  I  Y  Q  Q  V  R  D  E  L  K  R  A
1126 agtgtgtctcaagctgtctttgcaagagtggcattcaaccgcaca
     S  V  S  Q  A  V  F  A  R  V  A  F  N  R  T
1171 cagggattattgtcagagatactgcgtaaggaagaagatcccagg
     Q  G  L  L  S  E  I  L  R  K  E  E  D  P  R
1216 actgcgtctcagtctcttctagtaaacctgagggccatgcagaac
     T  A  S  Q  S  L  L  V  N  L  R  A  M  Q  N
1261 ttcctcaacctgcctgaagtggagcgtgatcgcatttaccaggat
     F  L  N  L  P  E  V  E  R  D  R  I  Y  Q  D
1306 gagcgagagaggagcatgaaccccaatgtgagcatggtctcctct
     E  R  E  R  S  M  N  P  N  V  S  M  V  S  S
1351 gcctctagcagtcccagctcctcccgaaccccacaggccaaaacc
     A  S  S  S  P  S  S  S  R  T  P  Q  A  K  T
1396 tcgacaccgacaacagacctccctattaaggtggacggcgccaac
     S  T  P  T  T  D  L  P  I  K  V  D  G  A  N
1441 gtcaacatcacagctgccatttatgacgagatccaacaggagatg
     V  N  I  T  A  A  I  Y  D  E  I  Q  Q  E  M
1486 aaaagagccaaggtgtctcaagccctgtttgccaaagtggctgca
     K  R  A  K  V  S  Q  A  L  F  A  K  V  A  A
1531 aacaaaagtcagggctggctttgcgaactgcttcgttggaaggag
     N  K  S  Q  G  W  L  C  E  L  L  R  W  K  E
1576 aaccccagcccagaaaaccgcacccttttgggagaatctctgcacc
     N  P  S  P  E  N  R  T  L  W  E  N  L  C  T
1621 atccgccgtttcctgaatcttccccaacatgagcgggatgtgatc
     I  R  R  F  L  N  L  P  Q  H  E  R  D  V  I
1666 tatgaggaagaatctcgacatcaccacagtgaacgcatgcagcat
     Y  E  E  E  S  R  H  H  H  S  E  R  M  Q  H
1711 gtggtccagctcccacctgagcccgtgcaggtccttcatcgacag
     V  V  Q  L  P  P  E  P  V  Q  V  L  H  R  Q
1756 cagtcccagccaactaaggagagctcccctcccagagaagaagca
     Q  S  Q  P  T  K  E  S  S  P  P  R  E  E  A
1801 cccccaccgcctcctccaacagaagacagctgtgccaaaaagcct
     P  P  P  P  P  P  T  E  D  S  C  A  K  K  P
1846 cggtctcgcacaaagatctctttggaagccctgggcatccttcaa
     R  S  R  T  K  I  S  L  E  A  L  G  I  L  Q
1891 agcttcatccatgatgtaggcctctatcccgaccaggaagccatc
     S  F  I  H  D  V  G  L  Y  P  D  Q  E  A  I
1936 cacacactctccgcccagctggatctccccaaacacaccatcatc
     H  T  L  S  A  Q  L  D  L  P  K  H  T  I  I
1981 aagttcttccagaaccagaggtaccacgtgaagcaccacgggaag
     K  F  F  Q  N  Q  R  Y  H  V  K  H  H  G  K
2026 ctgaaagagcacctgggctccgcggtggacgtggctgaatataag
     L  K  E  H  L  G  S  A  V  D  V  A  E  Y  K
2071 gacgaggagctgctgaccgagtcagaggagaacgacagcgaggaa
     D  E  E  L  L  T  E  S  E  E  N  D  S  E  E
2116 ggctccgaggagatgtacaaagtggaggctgaggaggaaaatgct
     G  S  E  E  M  Y  K  V  E  A  E  E  E  N  A
2161 gacaaaagcaaggcagcacctgccgaaattgaccagagataa 2202
     D  K  S  K  A  A  P  A  E  I  D  Q  R  *
```

FIGURE 9 SEQ ID NO:4 Mouse protein sequence

```
001     MERRSESPCL RDSPDRRSGS PDVKGPPPVK VARLEQNGSP MGARGRPNGA
051     VAKAVGGLMI PVFCVVEQLD GSLEYDNREE HAEFVLVRKD VLFSQLVETA
101     LLALGYSHSS AAQAQGIIKL GRWNPLPLSY VTDAPDATVA DMLQDVYHVV
151     TLKIQLQSCS KLEDLPAEQW NHATVRNALK ELLKEMNQST LAKECPLSQS
201     MISSIVNSTY YANVSATKCQ EFGRWYKKYK KIKVERVERE NLSDYCVLGQ
251     RPMHLPNMNQ LASLGKTNEQ SPHSQIHHST PIRNQVPALQ PIMSPGLLSP
301     QLSPQLVRQQ IAMAHLINQQ IAVSRLLAHQ HPQAINQQFL NHPPIPRAVK
351     PEPTNSSVEV SPDIYQQVRD ELKRASVSQA VFARVAFNRT QGLLSEILRK
401     EEDPRTASQS LLVNLRAMQN FLNLPEVERD RIYQDERERS MNPNVSMVSS
451     ASSSPSSSRT PQAKTSTPTT DLPIKVDGAN VNITAAIYDE IQQEMKRAKV
501     SQALFAKVAA NKSQGWLCEL LRWKENPSPE NRTLWENLCT IRRFLNLPQH
551     ERDVIYEEES RHHHSERMQH VVQLPPEPVQ VLHRQQSQPT KESSPPREEA
601     PPPPPPTEDS CAKKPRSRTK ISLEALGILQ SFIHDVGLYP DQEAIHTLSA
651     QLDLPKHTII KFFQNQRYHV KHHGKLKEHL GSAVDVAEYK DEELLTESEE
701     NDSEEGSEEM YKVEAEEENA DKSKAAPAEI DQR
```

TABLE 1: **Oligonucleotides used in the Study.**

Marker	Oligonucleotide Primers	YACs isolated	Chimeric by FISH	SEQ ID NO
D2S311	dCAATTTTGAGCCCGGAAG dTGACTAGAAAGGCATTCCAGAG	17GD1 31CH5 33AC9	+ - -	5 6
D2S115	dCAAGAACAGCCATATTGACTTGAAC dGGGTACAGCCCATGTGTGAG	11GG8	-	7 8
D2S348	dAGGTGACCAGCAGCCTCT dGTAAAACGGACATATCCCCC	19ID10 21GA12 23EG11 32EB9	ND ND ND ND	9 10
D2S72	dAGCTATAATTGCATCATTGCA dTGGTCTATAACGGTCTATG	26IF5	-	11 12
D2S105	dCTCTACAGTTTATAACCAGC dTACACTGGATTCATATTCCC	26IF5	-	13 14
C7LA4	dATTTCAATTTCCAAGAGCTGAGG dGCTGATGTGACAGAAACATCCC	26IF5 8IH5 13HC12 20AG2 22HB6	- ND ND ND ND	15 16
D2S307	dCATGACCTGAAATAAACATAGACA dDAGCTTTTCCTGTAGGCTGTC	26IF5 22HB6 6BC7	- ND ND	17 18
D2S1384	dAATAGAGGGCCCTTGCTTAA dTTTGGGATAAAAGGTATTTTGC	26IF5 22HB6 10GF2	- ND ND	19 20
WI5293	dGAGTTAGACCCCGTCTAAAAAAA dACTCTCATCTCCTTCCTTGTTCC	26IF5 8BG7 14HA2 24GF8	- ND - ND	21 22
D2S2189	dTACAAAAGGACTTGTCCAGGG dTCAAGATTGCCGTGAGGT	8BG7 14HAZ	ND -	23 24

		23CE7	ND	
		24GF8	ND	
D2S1271	dGGAAGGTCCAGATTAGAAG	15BA12	ND	25
	dAAGGGAAATAAAGAGAAGCAT	22HC8	ND	26
D2S116	dCAATCTCCACAAGTTGCTCA	6HA11	-	27
	dGGGATAGATAATTTAGGAGTGGG	13BE7	ND	28
		14DE4	ND	
		16IB4	+	
		28DE5	ND	
D2S309	dGCTCTAGTAGGCTGGTTACATAA	4EC12	+	29
	dTTCCAAGAATAATGCAATCTCAG	31DH5	+	30
FN1	dTTGTTCCTACAGTATTGCGGG	7AH3	+	31
	dCCAACCCAAGATGCAAATG	11GH11	ND	32
		31GG9	-	
		37HB8	ND	
IGFBP5	dCTATTGGGGTTTCCCAGGAT	21EC3	-	33
	dTTTCCAATATTGGGGCATGT	22DB10	ND	34
IGFBP2	dCAGTAGACCGCAGCCAGC	7FA11	ND	35
	dGGAAAGCAAGAAGGAGCAGG	8HF12	ND	36
		21EC3	-	
		22DB10	ND	
IHH	dGGACTCCACCTGGAACTGC	35EF10	-	37
	dGAAAACTCGTAGTGAGAGCAG			38

TABLE 2: Genetic Map of CP-1 Region

A	Cen	B	Cen	
	D2S311		D2S311	der(2)
			D2S374	⇐
			D2S1413	⇐
	D2S2327		D2S115	der(7)/der(11)
			D2S348	der(7)/der(11)
	D2S2396		D2S72	der(7)/der(11)
			(D2S2327)	
	D2S374		(D2S2396)	
	D2S2217		D2S105	der(7)/der(11)
			CTLA4	der(7)/der(11)
	1.4cM		D2S307	der(7)/der(11)
			(D2S2217)	
	D2S2392		(D2S2392)	
	0.1		(D2S1740)	
	cM			
			(D2S2708)	
	D2S309		(D2S1837)	
			(D2S2684)	
	Tel		(D2S1367)	
			D2S1384	der(7)/der(11)
			WIS293	der(7)/der(11)
			D2S2189	der(7)/der(11)
			D2S1271	der(7)/der(11)
			D2S116	der(7)/der(11)
			D2S309	der(7)/der(11)
			Tel	

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled WOUND HEALING AND OROFACIAL CLEFTING, the specification of which

☐ is attached hereto.

☒ was filed on 29 June 2001 as United States Application No. 09/869,564.

☒ was filed on 6 January 2000 as International Application No. PCT/GB00/00003.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
9900167.9	United Kingdom	6 January 1999	☒	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code,§ 119(e) of any United States provisional application(s) listed below:

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/GB00/00003	6 January 2000	Pending
(Application No.)	(Filing Date)	(Status: patented, Pending, abandoned)

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from Harrison Goddard Foote as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the practitioners associated with the customer number provided below to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SLR:SAS:nms:dm 08/17/01 5585-59367 59065.doc

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